

A Robust 384-Well Cell Migration Assay for High Content Analysis

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Abstract

We have developed a first-in-class 384-well cell migration assay to enable high throughput screening applications. The assay is completely automatable with liquid handling systems and high content image analysis instrumentation. This assay format utilizes a centrally located self-dissolving, non-toxic biocompatible gel (BCG) to form a uniformly sized, cell-free detection zone on tissue culture treated or collagen I coated cell culture surfaces. Cells are seeded into 384-well plates and pattern in an annular monolayer surrounding the BCG. Once the BCG dissolves, cells can migrate into the detection zone previously occupied by the BCG. Cell viability and cytotoxicity assays demonstrate that the dissolved BCG is non-toxic to commonly used cell lines including HT-1080, MDA-MB-231, PC-3 and human umbilical vein endothelial cells (HUVECs). This assay format allows an unobstructed view of cell motility throughout the duration of the experiment. Cells may be fixed and treated with multiple stains, including DAPI to visualize nuclei, propidium iodide to visualize dead cells, and TRITC-phalloidin to observe F-actin, to enable flexible data capture by either enumerating migrating cells or by calculating the area of closure within the detection zone to obtain robust Z' factors. Dose response experiments using the actin polymerization inhibitor Cytochalasin D, as well as other classes of migration inhibitors, were successfully automated using robotic liquid handlers and High Content Imaging (HCI) instruments.

Assay Schematic

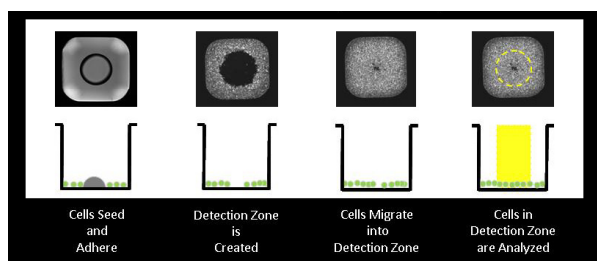


Figure 1. Schematic of Oris™ Pro 384 Cell Migration Assay.

Biocompatibility of BCG

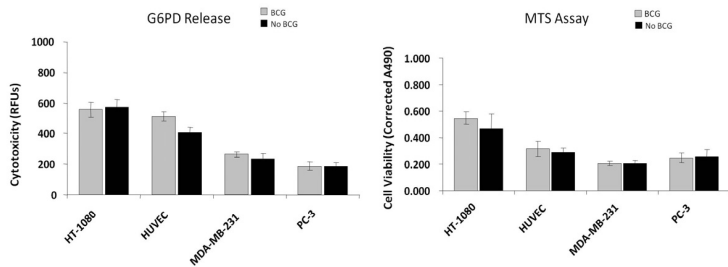


Figure 2. Effect of BCG on Cytotoxicity and Cell Viability using G6PD Release and MTS Assays. The Vybrant™ Cytotoxicity Assay (Molecular Probes) was used to measure the amount of glucose 6-phosphate dehydrogenase (G6PD) released from damaged cells and the CellTiter96® Aqueous One Solution Cell Proliferation Assay (Promega) was used to measure metabolic activity in live cells through the reduction of MTS substrate into formazan. Data represent average values and absorbance values, respectively, for these assays ± SD for 8 wells per condition as measured using a BioTek Synergy™ HT microplate reader. Results indicate no substantial cytotoxicity or viability effects from BCG on HT-1080 fibrosarcoma cell, human umbilical vein endothelial cell (HUVEC), MDA-MB-231 breast cancer cell and PC-3 prostate cancer cells.

Flexibility of Data Capture

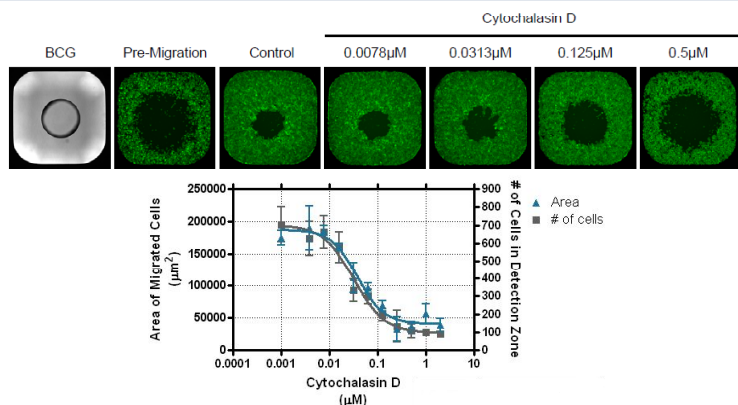


Figure 3. Effect of Cytochalasin D on HUVEC Migration. (A) Representative images of TRITC-phalloidin stained HUVEC cells in the presence and absence of Cytochalasin D (at indicated concentrations in μM) after 16 hr of migration as captured by a Zeiss Axiovert inverted microscope (pseudocolored green). (B) Dose-response curves as calculated by analyzing detection zones for area of migrated cells or number of cells using an Acumen™X3 scanning laser microplate cytometer (TTP LabTech). The calculated IC_{50} values for area and cell number determinations were 0.038 and 0.032 μM , respectively. Data represents the average of 4 replicates per treatment condition ± SD.

Image Acquisition Strategies for HCA

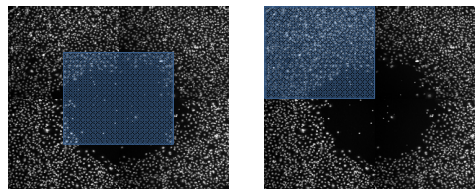


Figure 4. Image Acquisition and Strategies for Analysis of Cell Migration on Oris™ Pro 384 well Plates. Composite panels show Hoechst channel images of MDA-MB-231 cells acquired on a Cellomics ArrayScan II high content image analyzer (ThermoFisher) using a 5X objective. **Left panel**, a single image taken in the center of the well captures cells that have migrated into the detection zone. **Right panel**, a single imaging field positioned at the edge of the detection zone yields sufficient numbers of cells for analysis of cellular toxicity, morphology, and markers of compound activity. For subsequent experiments data were generated from a single imaging field.

Multiparametric Analysis of Compound Effects

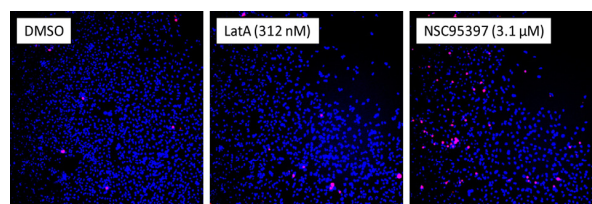


Figure 5. Evaluation of Cytotoxicity of Compounds on MDA-MB-231 Cells at Corresponding IC_{50} Values for Cell Migration. 48 h after treatment, cells treated with vehicle (0.5% DMSO) migrated into the detection zone (positioned in the upper right hand quadrant). Propidium iodide (PI) staining of live cells treated with DMSO shows low numbers of PI-positive cells (pink) documenting lack of cytotoxicity for both vehicle and BCG. Latrunculin A inhibited cell migration without altering cell permeability. In contrast, NSC95397 showed increased numbers of PI positive cells (pink). These data document different activity profiles for the inhibitors and suggest that cellular toxicity may contribute to the anti-migratory effect of NSC95397.

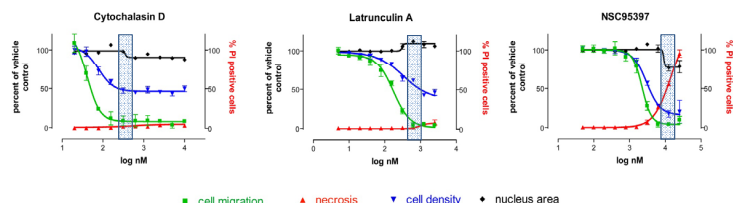


Figure 6. Multiparametric Measurements of Cell Migration and Toxicity. MDA-MB-231 cells (8,000/well in 15 μL) were seeded in Oris™ Pro 384 collagen I-coated plates. After a 2 h incubation to allow for attachment, seeding media was removed and cells were treated with ten point two-fold dilution gradients of the cytoskeleton-perturbing agents Cytochalasin D, Latrunculin A, or the multitargeted dual specificity phosphatase inhibitor, NSC95397. 48 h after treatment, cells were stained with 2.5 $\mu\text{g}/\text{mL}$ Hoechst 33342 and 1 $\mu\text{g}/\text{mL}$ propidium iodide for 15 min and imaged on an ArrayScan II HCS reader. Graphs show inhibition of cell migration (green), percentage of PI positive cells (red), cell density (blue), and changes in nuclear morphology (black). Data represent the average ± SEM of 4 replicates for each concentration of compound. The S/B ratio for this assay was 24.6. The IC_{50} values for cell migration were calculated to be 40 nM for Cytochalasin D, 195 nM for Latrunculin A, and 2.5 μM for NSC95397. Concentrations of cytoskeletal perturbing agents that fully inhibited cell migration (shaded rectangles) caused a modest change in cell density, but did not affect cell membrane permeability or nuclear morphology. In contrast, NSC95397 caused changes in cell membrane permeability, profound cell loss, and nuclear condensation.

Conclusions

- The Oris™ Pro 384 Cell Migration Assay is a robust, easily automatable assay suitable for high throughput screening (HTS) and high content analysis (HCA).
- The BCG used to form the cell-free detection zone does not effect the viability of and is not cytotoxic to commonly used cells including HT-1080, HUVEC, MDA-MB-231 and PC-3.
- Data from the Oris™ Pro 384 Cell Migration Assay may be captured using scanning laser microplate cytometers or high content image analyzers and can be analyzed as either area of migrated cells or number of cells in the detection zone.
- The Oris™ Pro 384 Cell Migration Assay allows for multiparametric analysis of compound effects such as changes to membrane permeability, cell density and nuclear morphology.

Acknowledgements

The authors would like to thank Dr. David Onley of TTP LabTech for the analysis of the Oris™ Pro 384 Cell Migration Assay data, shown in Figure 3, on the Acumen™X3.

This work was supported by grants R43GM090386 and R21CA147985 from the National Institutes of Health to Platypus Technologies, LLC.